

## Production and characterization of an alloplasmic and monosomic addition line of *Brassica rapa* carrying the cytoplasm and one chromosome of *Moricandia arvensis*

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Intergeneric hybridization was performed between *Moricandia arvensis* and four inbred lines of *Brassica rapa* following embryo rescue. Three F<sub>1</sub> hybrid plants were developed from three cross combinations of *M. arvensis* × *B. rapa*, and amphidiploids were synthesized by colchicine treatment. Six BC<sub>1</sub> plants were generated from a single cross combination of amphidiploid × *B. rapa* ‘Ko1-303’ through embryo rescue. One BC<sub>2</sub> and three BC<sub>3</sub> plants were obtained from successive backcrossing with *B. rapa* ‘Ko1-303’ employing embryo rescue. Alloplasmic and monosomic addition lines of *B. rapa* (Allo-MALs, 2n = 21) were obtained from backcrossed progeny of three BC<sub>3</sub> plants (2n = 21, 22 and 23) without embryo rescue. An alloplasmic line of *B. rapa* (2n = 20) degenerated before foliation on 1/2 MS medium due to severe chlorosis. Allo-MALs of *B. rapa* (2n = 21) showed stable male sterility without any abnormal traits in vegetative growth and female fertility. Molecular analyses revealed that the same chromosome and cytoplasm of *M. arvensis* had been added to each Allo-MAL of *B. rapa*. This Allo-MAL of *B. rapa* may be useful material for producing cytoplasmic male sterile lines of *B. rapa*.

**Key Words:** intergeneric hybridization, *Brassica rapa*, *Moricandia arvensis*, alloplasmic line, monosomic addition line, chlorosis, cytoplasmic male sterility.

### Introduction

*Brassica rapa*, composed of a number of subspecies, such as *chinensis*, *japonica*, *narinosa*, *oleifera*, *pekinensis* and *rapifera*, has been used as a vegetable and oilseed crop throughout the world. In particular, it is a very important vegetable crop in East Asia. Commercial seeds of *B. rapa* cultivars in the market largely depend on F<sub>1</sub> hybrid seeds because of their higher productivity and uniform traits. Most F<sub>1</sub> hybrid seeds are produced using self-incompatibility controlled by S-alleles. However, self-incompatibility is not always suitable for production of F<sub>1</sub> hybrid seeds because it can be unstable under some environmental conditions (Horisaki and Niikura 2004). On the other hand, F<sub>1</sub> hybrid seed production of radish (*Raphanus sativus*), which also belongs to the Cruciferae, is based on both self-incompatibility and cytoplasmic male sterility (CMS). The Ogura cytoplasm found in Japanese radish (Ogura 1968) is most widely used as the male sterile cytoplasm of radish, and has been modified for introduction in *Brassica* crops with a restorer gene (Heyn 1976). CMS, a maternally inherited trait coded by the mitochondrial genome, is a more useful characteristic for

stable F<sub>1</sub> hybrid seed production, and has been used in many crops (onion, maize and sugar beet, etc.).

In several *Brassica* crop species (*B. rapa*, *B. juncea* and *B. napus*), a number of male sterile cytoplasm from related *Brassica* species have been introduced aggressively through interspecific and intergeneric hybridization or protoplast fusion followed by successive backcrossing: *Diplotaxis muralis* (Hinata and Konno 1979), *Brassica tournefortii* (Mathias 1985), *Brassica oxyrrhina* (Prakash and Chopra 1988), *Diplotaxis siifolia* (Rao *et al.* 1994), *Trachystoma ballii* (Kirti *et al.* 1995), *Eruca sativa* (Matsuzawa *et al.* 1999), *Erucastrum canariense* (Prakash *et al.* 1998), *Diplotaxis catholica* (Pathania *et al.* 2003), *Enarthrocarpus lyratus* (Deol *et al.* 2003), *Diplotaxis eruroides* (Bhat *et al.* 2006) and *Diplotaxis berthautii* (Bhat *et al.* 2008). On the other hand, the cytoplasm of *Moricandia arvensis*, which was first reported as a C<sub>3</sub>-C<sub>4</sub> intermediate species, has also been introduced to *B. juncea* (Prakash *et al.* 1998) and *R. sativus* (Bang *et al.* 2002). Furthermore, the *B. juncea* CMS line has been approved for commercial cultivation by Indian Council for Agricultural Research in India, after overcoming chlorosis through chloroplast substitution (Kirti *et al.* 1998) and introgression of the Rf gene(s) from *M. arvensis* nucleus (Prakash *et al.* 1998). However, no alloplasmic *B. rapa* line carrying *M. arvensis* cytoplasm has been reported. A number of intergeneric hybrids between

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*M. arvensis* and *B. rapa* have been produced by embryo rescue (Takahata and Takeda 1990). However, no backcrossed progeny have been produced because of the low fertility encountered in their breeding. Bang *et al.* (2002) were able to obtain alloplasmic *R. sativus* carrying the *M. arvensis* cytoplasm and *M. arvensis* monosomic addition lines of *R. sativus* by overcoming barriers in intergeneric hybridization between *M. arvensis* and *R. sativus* and in the backcrossing of an F<sub>1</sub> hybrid to *R. sativus* through embryo rescue followed by chromosome doubling of the F<sub>1</sub> hybrid.

In the present study, we describe the production of an alloplasmic and monosomic addition line of *B. rapa* carrying the cytoplasm and one chromosome of *M. arvensis*, and discuss its morphologic, cytogenetic and molecular characteristics.

## Materials and Methods

### Plant materials

*Moricandia arvensis* (L.) DC. strain 'MOR-ARV-4' (2n = 28, MaMa) which is preserved in the Tohoku University Brassica Seed Bank, Japan was used as the pistillate parent. Four inbred lines of *Brassica rapa* L. (2n = 20, AA) were used as the pollen parent: ssp. *pekinensis* strain 'Ha1-W10', 'Ha1-W610', ssp. *perviridis* strain 'Ko1-303' and ssp. *rapifera* strain 'Ka1-302' provided by the Tohoku Seed Company, Japan. Moreover, *B. rapa* L. ssp. *perviridis* cv. 'Syosai', a commercial F<sub>1</sub> cultivar provided by Tohoku Seed Company, was employed for comparison of morphological and molecular characteristics.

### Production of intergeneric F<sub>1</sub> hybrids and their progeny by successive backcrossing

Intergeneric hybridization between *M. arvensis* and *B. rapa* was performed using conventional crossing followed by embryo rescue. Flower buds were emasculated one day before flowering, immediately pollinated with fresh pollen and then bagged for approximately one week. Ovary culture followed by embryo culture was carried out for embryo rescue, according to Bang *et al.* (1996a). The embryo rescue technique was used to produce not only F<sub>1</sub> plants but also BC<sub>1</sub>, BC<sub>2</sub> and BC<sub>3</sub> plants. In order to synthesize amphidiploids, 0.2% colchicine solution was applied to the apical meristem of some F<sub>1</sub> seedlings. The seeds obtained from BC<sub>3</sub> plants by backcrossing were sown on soil and on solidified 1/2 MS medium (Murashige and Skoog 1962) containing 500 mg/L casein hydrolysate, 3.0% sucrose and 1.1% agar.

### Morphological and cytogenetic investigation of intergeneric F<sub>1</sub> hybrids and backcrossed progeny (BC<sub>1</sub> to BC<sub>4</sub> plants)

Morphology of flowers and siliques of BC<sub>3</sub> plants and seedlings of BC<sub>4</sub> plants by backcrossing with *B. rapa* was characterized. Meiotic chromosome behavior in pollen mother cells (PMCs) and pollen tetrads were observed using the 1% acetic orcein smear method. Somatic chromosome

number in root tip cells was examined using the Feulgen stain squash method followed by 1% acetocarmine staining. Pollen fertility was ascertained by observing 1000 pollen grains after staining with 1% acetocarmine.

### Molecular biological analysis in BC<sub>4</sub> plants derived from BC<sub>3</sub> plants carrying the cytoplasm and chromosomes of *M. arvensis*

Total DNA of BC<sub>4</sub> plants and their parent plants was extracted according to the CTAB method (Doyle and Doyle 1987). In order to clarify the organelle genome, we performed PCR analysis using a primer pair specific to mitochondrial 32 genes, described in Nahm *et al.* (2005), and to the chloroplast intergenic region (forward primer; 5'GAA CGACGGGAATTGAACC3', reverse primer; 5'TGCTGT TGAGGCTCCATCTA3'), which was designed based on the sequence of *trnH* and *psbA* in *Arabidopsis thaliana* (accession number AP000423). The 10 µl reaction mixtures contained 100 ng genomic DNA, 0.5 µM primers, 0.2 mM dNTPs (Fermentas, Vilnius, Lithuania), 1 × DreamTaq buffer and 0.5 unit of DreamTaq DNA polymerase (Fermentas) in 0.2 ml tubes, and PCR was carried out using a Program Temp Control system PC-320 (ASTEC Co., Fukuoka, Japan). Amplification conditions using primer pairs specific to mitochondrial genes were 94°C for 5 min followed by 35 cycles of 94°C for 30 sec, 54°C for 30 sec and 72°C for 2 min and finally 72°C for 10 min. For the primer pair specific to the chloroplast intergenic region, PCR conditions were 94°C for 5 min followed by 45 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 1.5 min and finally 72°C for 10 min. PCR products were mixed with loading dye and applied to a 0.7% or 3.0% agarose gel. After electrophoresis, the gel was stained by EtBr and observed on a transilluminator. For random amplified polymorphic DNA (RAPD) analysis, polyacrylamide gel electrophoresis was performed according to Kaneko *et al.* (2001) with some modifications. RAPD-specific markers detectable using 400 random primers of 12-mer sequences (Common A to D, BEX, Tokyo, Japan) were employed for identification of the additional chromosome in BC<sub>4</sub> plants. The reaction mixture composition was as above except using 20 ng genomic DNA and 0.2 µM primer. Amplification conditions were as described in Akaba *et al.* (2009). After carrying out electrophoresis on a 0.5% polyacrylamide gel, the gel was viewed as above.

## Results

### Production and characterization of intergeneric F<sub>1</sub> hybrids and backcrossed progeny up to the BC<sub>3</sub> generation

In intergeneric hybridization between *M. arvensis* and four inbred lines of *B. rapa*, three amphidiploid plants (2n = 48, MaMaAA) were synthesized from three cross combinations through embryo rescue followed by colchicine treatment (Table 1). When the amphidiploid plants were backcrossed with the same inbred line of *B. rapa* as pollen

**Table 1.** Production of intergeneric hybrids between *Moricandia arvensis* and *Brassica rapa* and BC<sub>1</sub>, BC<sub>2</sub> and BC<sub>3</sub> plants by successive backcrossing with *B. rapa* using ovary culture followed by embryo culture

Generations	Cross combinations (2n)	No. of flowers pollinated	No. of ovaries cultured	No. of embryos cultured	No. of seeds obtained	No. of hybrid plants (2n)
F <sub>1</sub>	<i>M. arvensis</i> × 'Ka1-302'	184	28	0		–
	<i>M. arvensis</i> × 'Ko1-303'	74	20	2		1 (48) <sup>a</sup>
	<i>M. arvensis</i> × 'Ha1-W10'	150	10	1		1 (48) <sup>a</sup>
	<i>M. arvensis</i> × 'Ha1-W610'	162	30	6		1 (48) <sup>a</sup>
BC <sub>1</sub>	MaMaAA-1 (48) <sup>b</sup> × 'Ko1-303'	25	18	6		6 (34)
	MaMaAA-2 (48) <sup>c</sup> × 'Ha1-W10'	36	30	0		–
	MaMaAA-3 (48) <sup>d</sup> × 'Ha1-W610'	18	4	0		–
BC <sub>2</sub>	MaAA-BC <sub>1</sub> -1 (34) × 'Ko1-303'	103			0	–
	MaAA-BC <sub>1</sub> -2 (34) × 'Ko1-303'		140	2		1 (24)
BC <sub>3</sub>	BC <sub>2</sub> -1 (24) × 'Ko1-303'	118			20	0
		56	56	10		3 (21, 22, 23)

<sup>a</sup> The plant was synthesized to amphidiploids by colchicine treatment.

<sup>b</sup> MaMaAA-1 was amphidiploid F<sub>1</sub> plant between *M. arvensis* and 'Ko1-303'.

<sup>c</sup> MaMaAA-2 was amphidiploid F<sub>1</sub> plant between *M. arvensis* and 'Hal-W10'.

<sup>d</sup> MaMaAA-3 was amphidiploid F<sub>1</sub> plant between *M. arvensis* and 'Hal-W610'

parent of each amphidiploid, six BC<sub>1</sub> plants (2n = 34, MaMaAA) were derived, but only from the amphidiploid plant (MaMaAA-1) backcrossed with 'Ko1-303' through embryo rescue. Only one BC<sub>2</sub> plant (2n = 24) was generated from backcrossing of the BC<sub>1</sub> plants to 'Ko1-303' by embryo rescue; none were generated through conventional crossing. In production of BC<sub>3</sub> plant through successive backcrossing, no BC<sub>3</sub> plant developed from 20 seeds obtained by conventional crossing. However, three BC<sub>3</sub> plants developed from ten embryos cultured by embryo rescue; their somatic chromosome numbers were 2n = 21, 22 and 23, respectively (Table 1).

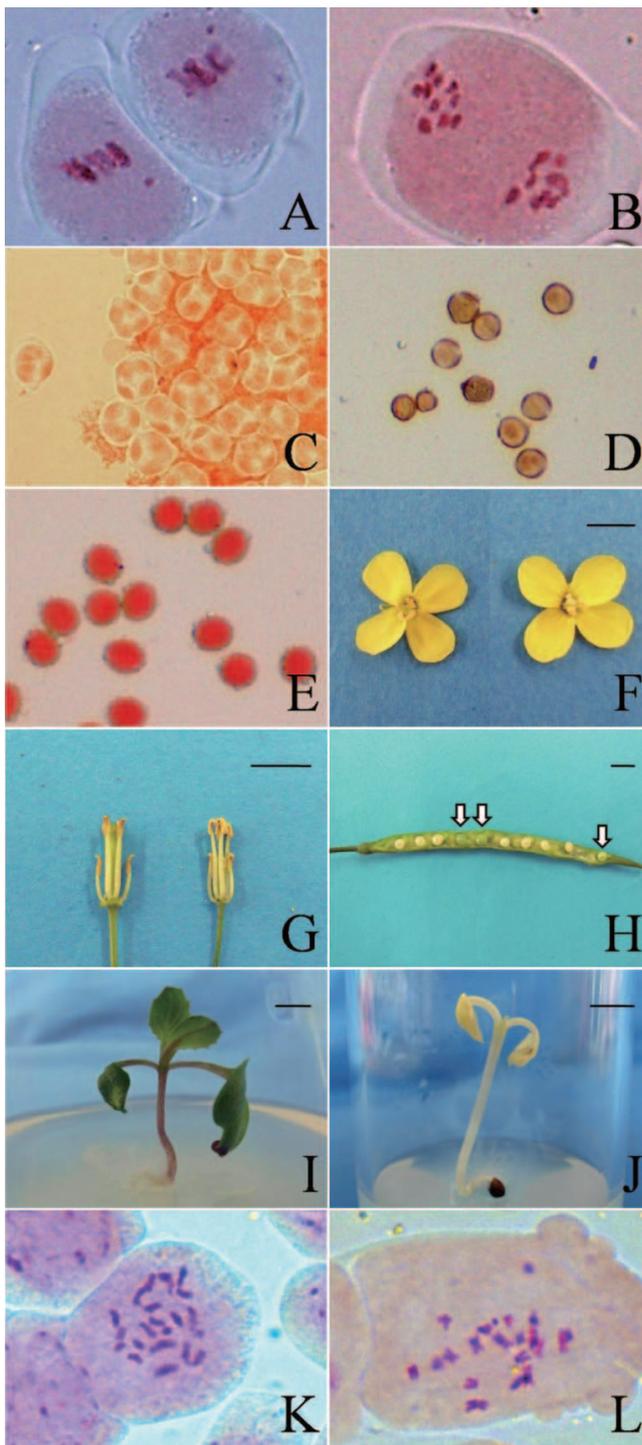
Meiotic chromosome configuration in PMCs of BC<sub>3</sub>-1 (2n = 21) was 10<sub>II</sub> + 1<sub>I</sub> in metaphase I and 10 + 11 in metaphase II (Fig. 1A, 1B). A BC<sub>3</sub>-1 (2n = 21) plant showed brown anthers with a few sterile pollen grains though formation of normal pollen tetrads (Fig. 1C–1G). There were few other differences in floral morphology between BC<sub>3</sub>-1 (2n = 21) and *B. rapa* 'Syosai'. On the other hand, the ovules of BC<sub>3</sub>-1 (2n = 21) formed both green and white embryos when backcrossed with *B. rapa* 'Ko1-303' (Fig. 1H).

#### Production and characterization of the Allo-MAL of *B. rapa* (Allo-MAL, 2n = 21) derived from BC<sub>3</sub> plants by backcrossing

Seeds obtained from three BC<sub>3</sub> plants by backcrossing were sown on soil in pots or on 1/2 MS medium. When the BC<sub>4</sub> seeds were sown on soil, their germination rate showed approximately 50% in each line which was generated from the BC<sub>3</sub> plants with chromosome number 2n = 21 and 2n = 22, and all progenies showed chromosome number 2n = 21 (Table 2). On the other hand, when the rest BC<sub>4</sub> seeds were sown on 1/2 MS medium, green and white seedlings were developed and their chromosome numbers showed 2n = 21 and 2n = 20, respectively (Table 2 and Fig. 1I–1L). Green seedlings grew well without any abnormality in vegetative growth but white seedlings degenerated

before foliation because of severe chlorosis. Accordingly, the low germination rate on soil seemed to be caused by the severe chlorosis which is appeared on the plant with chromosome number 2n = 20.

In PCR analysis using forward primer for *atp6* and reverse primer for *rrn18*, a marker of approximately 6000 bp specific to *M. arvensis* was detected in both green and white seedlings produced from backcrossing of BC<sub>3</sub>-1 (2n = 21) and BC<sub>3</sub>-2 (2n = 22) to *B. rapa* 'Ko1-303' (Fig. 2A), although the PCR product was not sequenced. No other markers specific to *M. arvensis* was detected from the primer pairs to mitochondrial 32 genes. Furthermore, using a primer pair specific to the chloroplast intergenic region, a major marker (about 400 bp) in addition to two minor markers specific to *M. arvensis* was observed in both green and white seedlings (Fig. 2B). This is clear evidence that both green and white seedlings in BC<sub>4</sub> generation are alloplasmic lines of *B. rapa* carrying *M. arvensis* cytoplasm. On the other hand, 17 RAPD markers specific to *M. arvensis* were detected by 14 random primers in each green seedling (2n = 21) produced from backcrossing of BC<sub>3</sub>-1 (2n = 21) and BC<sub>3</sub>-2 (2n = 22) with *B. rapa* 'Ko1-303', such as B09<sub>700bp</sub>, B41<sub>620bp</sub>, B41<sub>520bp</sub>, B43<sub>920bp</sub>, B44<sub>1000bp</sub>, B63<sub>620bp</sub>, B67<sub>920bp</sub>, B71<sub>1200bp</sub>, C35<sub>700bp</sub>, D06<sub>1400bp</sub>, D15<sub>820bp</sub>, D22<sub>700bp</sub>, D76<sub>4000bp</sub>, D76<sub>920bp</sub>, D77<sub>720bp</sub>, D77<sub>210bp</sub> and D90<sub>1100bp</sub> but these markers were not detected in white seedlings (Fig. 2C). When the other random primers were employed, there was not polymorphism between green and white seedlings. Therefore, molecular analyses showed that all Allo-MALs of *B. rapa* (green seedlings, 2n = 21) carried the cytoplasm and the same chromosome of *M. arvensis*, while white seedlings (2n = 20) carried the cytoplasm of *M. arvensis* with no extra chromosomes from *M. arvensis*.



**Fig. 1.** Morphological and cytogenetical characteristics of BC<sub>3</sub> and BC<sub>4</sub> plants obtained by successive backcrossing with *B. rapa*. A and B; chromosome behavior in Metaphase I ( $10_{II} + 1_I$ ) and Metaphase II ( $10 + 11$ ), respectively. C; Pollen tetrads, D; Sterile pollen grains of BC<sub>3</sub>-1 ( $2n = 21$ ). E; Fertile pollen grains of *B. rapa* 'Syosai'. F and G; Floral morphology of BC<sub>3</sub>-1 (left) and *B. rapa* 'Syosai' (right). H; Green (arrows) and white ovules existing in the silique of the BC<sub>3</sub>-1 by backcrossing with *B. rapa*. I and J; Green (I) and white seedlings (J) after two weeks of sowing. K and L; Somatic chromosome numbers of green seedling ( $2n = 21$ ) and white seedling ( $2n = 20$ ), respectively. Bars indicate 0.5 cm.

## Discussion

Some wild relatives in the Cruciferae have been evaluated as genetic resources for potentially useful agricultural traits providing biotic and abiotic resistance (Warwick, 1993). Therefore, diverse wide crosses between *Brassica* crops and wild relatives have been carried out (Kaneko *et al.* 2009). One of these wild relatives, *M. arvensis*, has useful characteristics of a C<sub>3</sub>-C<sub>4</sub> intermediate trait and CMS (Apel *et al.* 1978, Prakash *et al.* 1998). Sexual and somatic hybridization between *M. arvensis* and cruciferous crops employed as pollen parents or parents in cell fusion have been carried out to introduce the CMS trait of *M. arvensis* cytoplasm to *B. rapa* (Takahata and Takeda 1990), *B. nigra* (Takahata and Takeda 1990), *B. oleracea* (Takahata 1990, Ishikawa *et al.* 2003, Bang *et al.* 2007), *B. juncea* (Kirti *et al.* 1992, Takahata *et al.* 1993), *B. napus* (Takahata *et al.* 1993) and *R. sativus* (Bang *et al.* 1996b). However, only two alloplasmic lines of *B. juncea* and *R. sativus* carrying *M. arvensis* cytoplasm have been reported (Bang *et al.* 2002, Prakash *et al.* 1998).

In the present study, intergeneric hybrids between *M. arvensis* and *B. rapa*, and their backcrossed progeny up to the BC<sub>3</sub> generation, were produced using embryo rescue (Table 1). However, alloplasmic lines of *B. rapa*, in which the nuclear genome was completely substituted with *B. rapa*, were not able to reproduce. Three plants in the BC<sub>3</sub> generation showed  $2n = 21$ , 22 and 23, and seemed to have one to three chromosomes of *M. arvensis* added to the *B. rapa* chromosome number ( $2n = 20$ ) based on meiotic chromosome behavior in PMCs (Table 1 and Fig. 1A, 1B). After the BC<sub>3</sub> generation, each backcrossed progeny was able to reproduce through conventional crossing without embryo rescue, and showed chromosome number  $2n = 21$ , with only one chromosome of *M. arvensis* added to *B. rapa* ( $2n = 20$ ) (Table 2). Alloplasmic lines of *B. rapa* ( $2n = 20$ ) were only obtained after sowing on 1/2 MS medium, but they degenerated without foliation due to severe chlorosis (Table 2 and Fig. 1J, 1L). The siliques obtained from BC<sub>3</sub> plants ( $2n = 21$ ) by backcrossing to *B. rapa* formed both green and white ovules (Fig. 1H), and the Allo-MAL of *B. rapa* (Allo-MAL,  $2n = 21$ ) also formed two kinds of ovules after BC<sub>4</sub> generation. We concluded that the Allo-MALs of *B. rapa* ( $2n = 21$ ) and the alloplasmic *B. rapa* lines ( $2n = 20$ ) were generated from green and white ovules, respectively, and they were formed according to the theoretical value because the formation rate of Allo-MAL of *B. rapa* ( $2n = 21$ ) in the BC<sub>4</sub> generation was approximately fifty percent.

Each result of the cytogenetic and molecular analyses in this study revealed that the Allo-MALs of *B. rapa* ( $2n = 21$ ) generated from two BC<sub>3</sub> plants having chromosome numbers  $2n = 21$  and 22 all contained *M. arvensis* substitution cytoplasm and an identical chromosome from *M. arvensis*. In addition to that, the white seedlings with chromosome number  $2n = 20$  generated from the BC<sub>3</sub> plants by backcrossing to *B. rapa* were alloplasmic lines of *B. rapa* without any additional chromosome. Consequently, the alloplasmic

**Table 2.** Production of BC<sub>4</sub> plants derived from BC<sub>3</sub> plants (2n = 21, 22 and 23) by sowing on soil and medium, and their morphological and cytogenetical characteristics

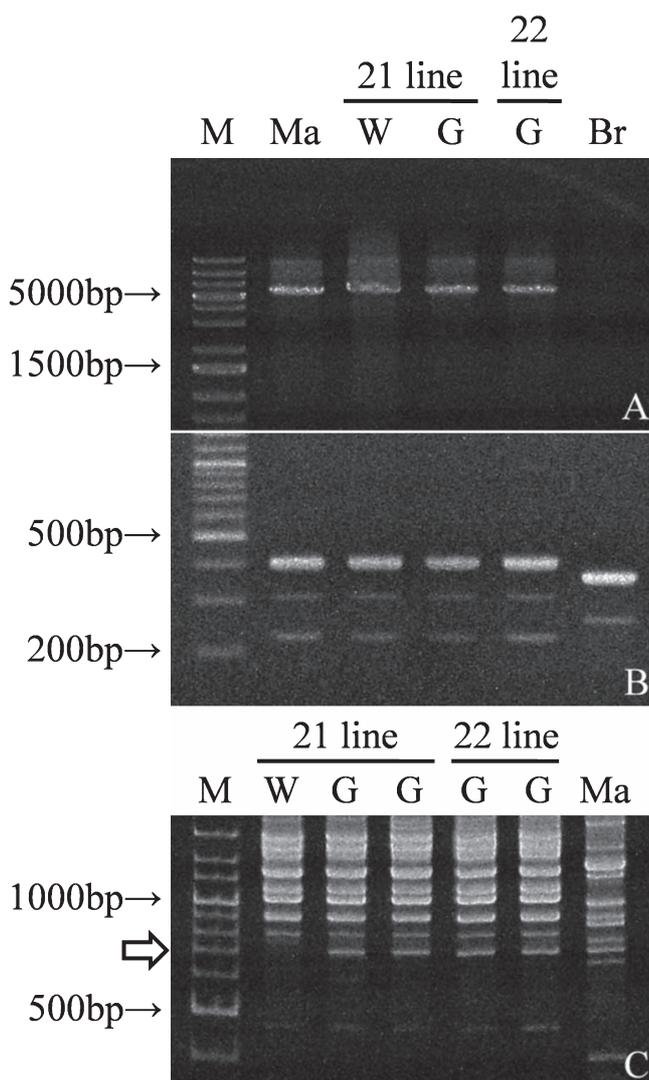
Cross combinations (2n)	No. of seeds obtained	Place of sowing	No. of seeds sown	Color of seedling				
				White No. of seedlings	Green No. of BC <sub>4</sub> plants (2n)			
					20	21	22	23
BC <sub>3</sub> -1 (21) × 'Ko1-303'	155	Soil	50	0	0	26	–	–
		Medium <sup>a</sup>	40	26	0	2	–	–
BC <sub>3</sub> -2 (22) × 'Ko1-303'	81	Soil	40	0	0	23	0	–
		Medium <sup>a</sup>	20	0	0	2	0	–
BC <sub>3</sub> -3 (23) × 'Ko1-303'	12	Soil	12	0	0	3	0	0

<sup>a</sup> 1/2 MS medium

*B. rapa* substituted with *M. arvensis* cytoplasm was not able to survive due to severe chlorosis, but the additional chromosome in the Allo-MALs of *B. rapa* (2n = 21) seemed to maintain the gene(s) for overcoming the chlorosis. An alloplasmic *R. sativus* line carrying *M. arvensis* cytoplasm, which was produced in the BC<sub>2</sub> generation through successive

backcrossing of an intergeneric hybrid between *M. arvensis* and *R. sativus*, showed light green leaves (Bang *et al.* 2002). On the other hand, an alloplasmic *B. juncea* line carrying the chloroplast and mitochondria genomes of *M. arvensis*, which was obtained in the BC<sub>3</sub> generation following backcrossing of a somatic hybrid between *B. juncea* and *M. arvensis*, showed yellowish leaves (Prakash *et al.* 1998). As mentioned above, no alloplasmic *B. rapa* substituted with *M. arvensis* cytoplasm was obtained up to the BC<sub>4</sub> generation due to severe chlorosis. Accordingly, the varying degrees of leaf chlorosis occurring in the alloplasmic lines substituted with the *M. arvensis* cytoplasm seemed to originate from incongruous interaction between the chloroplast genome of *M. arvensis* and the nuclear genome of the recipient species, and to be closely related to the female sterility in backcross progeny of interspecific and intergeneric hybridizations.

The Allo-MALs of *B. rapa* (2n = 21) we obtained showed stable male sterility without any abnormal traits in vegetative growth or female fertility. The flowers were normal except for slender anthers containing only sterile pollen and excellent nectaries (Fig. 1F, 1G). The pollen degeneration of Allo-MALs of *B. rapa* (2n = 21) seemed to occur at the microspore based on normal chromosome behavior and formation of normal pollen tetrads (Fig. 1A–1D). The *M. arvensis* cytoplasm may be a novel CMS source in *B. rapa* in addition to *B. juncea* and *R. sativus*. However, the CMS system in *B. rapa* may be not used without overcoming the severe chlorosis. Kirti *et al.* (1998) was able to overcome chlorosis occurring in alloplasmic *B. juncea* carrying *M. arvensis*



**Fig. 2.** Molecular analyses in BC<sub>4</sub> plants obtained from BC<sub>3</sub> plants with chromosome numbers 2n = 21 and 2n = 22 by backcrossing to *B. rapa*. PCR analysis using primer pairs specific to mitochondrial genes (A) and chloroplast intergenic region (B) and RAPD analysis using Common D22 as random primer (C). Primer pairs in A are forward primer for *atp6* and reverse primer for *rrm18*. Primer pair in B is specific to intergenic region between *trnH* and *psbA*. The 21 and 22 lines are backcrossing progenies derived from BC<sub>3</sub>-1 (2n = 21) and BC<sub>3</sub>-2 (2n = 22), respectively. Ma; *M. arvensis*. W; White seedling (2n = 20). G; Green seedling (2n = 21). Br; *B. rapa* 'Syosai'. M; Size marker. Arrow indicates marker specific to *M. arvensis*.

cytoplasm through replacement of the chloroplasts of alloplasmic *B. juncea* with those of normal green *B. juncea* by protoplast fusion. As indicated by Namai (1987), the introgression of desirable genes from one species to another of the Cruciferae has been accomplished via meiotic allo-synthesis in *Brassica* crops. Such homoeologous chromosome pairing is a practical method for intergenomic introgression. For example, the restorer genes for alloplasmic CMS *B. juncea* carrying the cytoplasm of *D. catholica*, *M. arvensis* or *T. ballii* were introduced by homoeologous recombination between chromosomes of *B. juncea* and cytoplasmic donors (Kirti *et al.* 1997, Pathania *et al.* 2003, Prakash *et al.* 1998). Takahata and Takeda (1990) reported that allosyndesis was observed in amphihaploid plants ( $2n = 24$ , MaA) between *M. arvensis* and *B. rapa*. If the alloplasmic *B. rapa* line ( $2n = 20$ ) with normal green leaves is produced by backcrossing progeny of Allo-MAL of *B. rapa* ( $2n = 21$ ) through homoeologous chromosome pairing, it might be a novel method for overcoming chlorosis in alloplasmic CMS lines carrying the *M. arvensis* cytoplasm. Therefore, the Allo-MAL of *B. rapa* ( $2n = 21$ ) obtained through successive backcrossing of intergeneric hybrids between *M. arvensis* and *B. rapa* may be useful breeding material for producing a cytoplasmic male sterile line of *B. rapa* having green leaves.

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